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Trichogramma chilonis Ishii: a potential biological control agent of *Crocidolomia pavonana* in Samoa

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Abstract

Crocidolomia pavonana F. (Lepidoptera: Crambidae) is a major pest of *Brassica* crops in tropical and sub-tropical regions of Africa, Asia and the Pacific. There are no previous reports of effective natural enemies of the pest across this range but in Samoa an arrhenotokous population of the generalist egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) frequently attacks it. This is the first record of *T. chilonis* in Samoa. A three-year field recruitment study showed that although *C. pavonana* eggs occurred at all times of the year, their abundance was greatest during drier periods. Parasitism of *C. pavonana* egg masses by *T. chilonis* was variable (0-87% of egg masses attacked) but the parasitoid was recovered from eggs collected at all times of the year and it is well established in the major *Brassica* growing regions of the island of Upolu. When partial lifetables were constructed for *C. pavonana*, the rate of egg disappearance (likely due to predation and the physical effects of rainfall) ranged from 0 to 0.839 and the marginal rate of mortality due to *T. chilonis* ranged from 0 to 0.474. When it was present, *T. chilonis* was the major mortality factor affecting *C. pavonana* eggs in all but one of the recruitment studies. The historical problems surrounding the identity and species status of *T. chilonis* are discussed and its host range and distribution in the Asia- Pacific region is reviewed briefly. Finally, the potential of this population of *T. chilonis* for development as a biological control agent of *C. pavonana* is considered.

Keywords: integrated pest management, Brassica, egg parasitoid, diamondback moth, Pacific

1. Introduction

Worldwide, approximately 86 million tonnes of *Brassica* vegetable crops are grown annually (FAOSTAT, 2013). These crops are attacked by a wide range of pest Lepidoptera and in tropical and sub-tropical regions of Africa, Asia and Oceania *Crocidolomia pavonana* (F.) (Lepidoptera: Crambidae) is a major constraint to production (Morallo-Rejesus and Navasero-Ward, 2003; Karungi et al., 2010; Muniappan and Marutani, 1992; Sastrosiswojo and Setiawati, 1992; Shepard and Schellhorn, 1997; Srinivasan and Veeresh, 1986). The pest is considered to be native to Asia and Africa (Waterhouse and Norris, 1987), but it is now widely distributed through tropical and subtropical regions of central and southern Africa, Madagascar, south, southeast and east Asia, the Indian Ocean, eastern Australia and the islands of the South Pacific (CABI, 2013).

Crocidolomia pavonana lays eggs at night in egg masses of variable sizes (10->100 eggs) and when reared on head cabbage (*Brassica oleracea* L. var. *capitata*) female fecundity has been estimated at 350 eggs (Sastrosiswojo and Setiawati, 1992). Eggs are usually laid at the edge or margin of the lower surface of foliage and, prior to head formation, ≈ 90% of egg masses are laid on the upper leaves of plants (Takeuchi et al., 2009). Upon hatching, larvae feed gregariously near the site of oviposition before moving towards the plant meristem where they feed under silk webbing on the growing tips or on the young unexpanded leaves (Takeuchi et al., 2009). This feeding pattern can result in the destruction of young plants, rendering attack by *C. pavonana* a particularly serious problem (Takeuchi et al., 2009). All stages of host plants are susceptible to attack by *C. pavonana* and major yield losses can occur if the pest population is not

suppressed (Morallo-Rejesus and Navasero-Ward, 2003). Although immature plants are especially susceptible to *C. pavonana* attack, single larvae can destroy more mature plants by mining into the heads (Morallo-Rejesus and Navasero-Ward, 2003; Smyth et al., 2003). When larval development is complete, insects either pupate within folds in host plant leaves or, more commonly, pre-pupae move to the soil surface before burrowing to establish pupation chambers to depths of up to 10 cm below the surface (Singh and Rawat, 1983).

Crocidolomia pavonana is relatively susceptible to most commonly used insecticides, and the widespread use of synthetic insecticides following the Second World War diminished its status as the principal insect pest of *Brassica* crops in tropical and sub-tropical regions of the Old World (Ankersmit, 1953). Throughout its host range *C. pavonana* frequently co-occurs with the serious *Brassica* pest *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Furlong et al., 2013). Effective management strategies based on the adoption of selective insecticides and the establishment of solitary larval parasitoids (*Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) in temperate and highland tropical regions and *Cotesia vestalis* Halliday (Hymenoptera: Braconidae) in lowland regions of the tropics) have been developed for *P. xylostella* (Furlong et al., 2013). This complicates management, as although appropriately targeted selective insecticides can effectively suppress *C. pavonana* populations, the use of non-selective compounds can severely disrupt the natural enemies of *P. xylostella*, leading to resurgence of this severe pest. The emphasis on reduction of broad-spectrum insecticide use in *Brassica* crops to promote *P. xylostella* biological control (Furlong et al., 2004a,b) has renewed interest in *C. pavonana* field biology and ecology in the tropics

as integrated strategies for the management of the lepidopteran pest complexes attacking *Brassica* vegetable crops are desperately required.

The natural enemies attacking *C. pavonana* have not been well documented anywhere in its range, and although there have been reports of larval parasitoids attacking the pest in the Philippines (Barrion et al., 2003), Indonesia (Shepard and Schellhorn 1997), Papua New Guinea (Saucke et al., 2000), India (Singh and Rawat, 1980) and southern Africa (Gunn, 1925; Smee, 1942) parasitism rates are invariably low, and pest populations are not suppressed satisfactorily. Similarly, although egg parasitoids have been recorded attacking *C. pavonana* in the Philippines (Morallo-Rejesus et al., 1996), Indonesia (Shepard and Schellhorn, 1997; Buchori et al., 2010) and Papua New Guinea (Saucke et al., 2000), the associations appear to be incidental and parasitism rates are very low.

In this study we record the generalist egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) attacking *C. pavonana* in the field in Samoa. This is the first record of egg parasitism of *C. pavonana* in Samoa, and only the second reported association between these two species; Saucke et al. (2000) reared a single *T. chilonis* from *C. pavonana* in Papua New Guinea. The impact of this population of *T. chilonis* on *C. pavonana* egg populations was investigated in field recruitment studies conducted over a three-year period, and extensive surveys recorded the incidence of the pest and parasitoid in the main *Brassica* growing regions of Upolu island, Samoa. The potential of the parasitoid for development as a biological control agent is discussed, and the complicated issues of *T. chilonis* species identity, status and distribution are reviewed briefly.

2. Materials and methods

2.1 Experimental field sites

All experiments were conducted at Nu'u Crops Research Center (13° 49' S, 171° 50' W) on the island of Upolu, Samoa between July 2006 and December 2009. Head cabbage (*Brassica oleracea* var. *capitata* cv. KK cross) plants for use in field experiments were grown from seed in a mixture of field soil and organic compost and maintained in a shade-house and transplanted to the field at the 5-leaf stage. Plants were arranged in single rows and spaced 0.5 m apart; adjacent rows were spaced 0.75 m apart. Seedlings were fertilized (N: P: K; 12: 5: 20) at the time of transplanting and subsequent applications of fertilizer were made four and eight weeks later. Plants were hand watered as necessary. Single field plots consisted of 600- 1000 plants and 8-10 weeks after seedlings were transplanted another plot was established nearby (≤ 100 m away). Plots were typically maintained for 12 weeks after transplanting, which enabled a continuous crop to be maintained at the research station for most of the duration of the study.

Throughout the study daily rainfall and maximum and minimum temperatures were recorded at a nearby weather station.

2.2 Pest and parasitoid sampling

Experimental plots at Nu'u Crops Research Center were sampled at least two times per week from transplanting until they were 10-12 weeks old; on occasions the planting of the new crop was delayed and in these circumstances the original crop was maintained

for up to 14 weeks after transplanting. There were short periods when no crop was grown. On each sampling occasion 30-60 plants were carefully inspected by hand and the number of *C. pavonana* egg masses and first instar larvae on each inspected plant recorded.

Between June 2011 and November 2013 cabbage and Chinese cabbage crops on farms in the main *Brassica* growing region of Aleisa, in the area close to Nu'u Crops Research Center and on the north coast of Upolu were periodically surveyed for *C. pavonana*. At each survey site 100-300 plants were examined and all *C. pavonana* egg masses were collected and taken back to the laboratory for rearing to determine if they were parasitized.

2.3 Egg parasitoid collection and identification

During the experiments at Nu'u, *C. pavonana* egg masses were periodically collected from the field and held singly in Petri dishes (9cm diameter) until larvae hatched or blackened eggs appeared. Egg masses containing blackened eggs were then transferred individually to gelatin capsules (20mm x 7.5mm) until adult parasitoid wasps emerged. Ten individual wasps that were reared from ten discrete field-collected *C. pavonana* egg masses (eight egg masses field-collected in late 2007 and two egg masses field-collected in September 2013) were squashed onto separate Whatman® FTA® PlantSaver Cards (Whatman Inc., Florham Park, NJ). The cards were dried and then shipped under ambient temperatures to the University of California, Riverside, USA for identification. Each individual wasp was removed from the FTA® card as a 1.2 mm diameter disc, which was subsequently transferred to a 0.2 mL PCR tube. Individual discs were incubated in

200 µL FTA® Purification Reagent for 5 min at room temperature, and then washed with 200 µL of $TE_{0.1}$ for a further 5 min, again at room temperature. FTA® discs were then allowed to air dry for at least 30 min before being used directly as template in the PCR reaction. The ITS2 spacer of the wasps was amplified using the primers and reaction conditions described by Stouthamer et al. (1999). PCR products were purified and direct sequenced at the Genomics Core of the University of California, Riverside. The ITS2 sequences of the specimens were then compared with the known sequences present in GenBank using BLAST.

Adult male wasps that emerged from egg masses collected in late 2007 were examined microscopically. Specimens were placed in an aqueous mixture of glacial acetic acid, lactic acid and phenol for 6- 12 h to clarify them (Lin, 1994). They were then transferred to a cavity microscope slide, mounted in Hoyer's medium, and under a stereomicroscope, the body wall was removed with a fine needle to expose the genitalia. Mounted specimens were covered with a glass coverslip and examined under a compound microscope (x1000 magnification); images were taken with a digital camera (Canon G10 (14.7 megapixels, x5 optical zoom).

2.4 Recruitment of C. pavonana *and* T. chilonis

Detailed mapping of *C. pavonana* egg masses was conducted in experimental plots at Nu'u on 7 occasions between September 2007 and December 2009. Each plant in a defined grid of plants (n=36-380) within an experimental plot was searched thoroughly for *C. pavonana* egg masses. Each egg mass that was discovered was marked and its location on the plant and the location of the plant within the grid of plants selected for

the survey were recorded. All plants within the survey grid were then revisited on successive days, new egg masses were marked and the status of previously marked egg masses was recorded. Three days after they were marked, individual egg masses were placed into containers and returned to the laboratory for rearing to assess mortality and parasitism. The number of eggs recruited to the population during the study was estimated by multiplying the mean number of eggs per surviving egg mass by the total number of egg masses sampled on experimental plants; total egg disappearance was estimated in a similar manner. The number of eggs parasitized by *T. chilonis* was measured directly by counting the number of parasitized eggs in each egg mass after rearing in the laboratory. Marginal rates of egg disappearance and *T. chilonis* parasitism were calculated using the methods described by Furlong et al. (2004a).

In December 2009, the adult parasitoids that emerged from 10 field collected egg masses were collected. Each parasitoid cohort was examined separately under a binocular light microscope (Meiji, x10) and the number of male and female wasps present in each was determined based on antennal structure.

3. Results

3. 1 Pest and parasitoid sampling

Crocidolomia pavonana egg masses were recorded in all cabbage crops planted for the study at Nu'u (Fig. 1). The density of egg masses varied widely, from a mean of 28.6 (±15.6) egg masses per 60 plants in August 2006 to a mean of 0.25 (±0.46) in April 2008;

densities were typically higher in the drier months of May- October than in the wetter months of November- April (Fig. 1)

In *Brassica* farm investigations conducted 2011-2013, *T. chilonis* was reared from *C. pavonana* collected in all three regions surveyed (Fig. 2). In the 28 surveys conducted *T. chilonis* was reared from egg masses collected on 17 occasions and up to 87% of egg masses were attacked (Fig. 2). Most surveys were conducted during peak *Brassica* production times (June- October), when *C. pavonana* densities are typically higher, but *T. chilonis* was also reared from *C. pavonana* egg masses collected from crops in January and April (Fig. 2).

3.2 Egg parasitoid collection and identification

All ten parasitoid specimens sequenced had identical ITS2 DNA sequences that belonged to the species *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae). Similarly, the morphology of the male genitalia (Fig. 3) was typical of *T. chilonis* (Nagarkatti and Nagaraja, 1971, 1977, 1979).

3.3 Recruitment of C. pavonana *and* T. chilonis

In the seven field studies conducted between September 2007 and December 2009 (Fig. 1), *T. chilonis* was recovered from field collected *C. pavonana* egg masses on five occasions (Table 1). In these studies, the marginal rate of egg disappearance ranged from 0- 0.839 and the marginal mortality due to *T. chilonis* parasitism ranged from 0-0.474 (Table 1). Across all these studies, total egg mortality rates ranged from 0.147-0.847 (Table 1).

In the 10 egg masses examined in the laboratory the female: male sex ratio ranged from 2.33: 1 to 10:1; overall the female: male sex ratio was 5.4: 1 (n= 128 adult parasitoids).

4. Discussion

Crocidolomia pavonana egg masses were recovered throughout the study, but their density fluctuated significantly over time and, in general, higher numbers of egg masses were found during drier periods (May- October) than during wetter periods of the year (November- April) (Fig. 1). In the two and a half year farm surveys (2011-2013) *C. pavonana* egg masses and *T. chilonis* were also recovered from all regions studied and the parasitoid was recovered in January, April and all months from June through to December (Fig 2.). This indicates that *T. chilonis* is well established in the *Brassica* growing regions of Upolu and that it can attack the pest during periods when crop production is low and *C. pavonana* host densities are typically reduced (Fig 1). *Trichogramma chilonis* is highly susceptible to insecticides (Wang et al., 2012), the use of which on *Brassica* farms in Samoa is typically very high. Both the incidence of the parasitoid and its impact on pest populations would likely increase significantly if insecticide use in *Brassica* production could be reduced through the adoption of selective insecticides and more integrated approaches to pest management.

In the recruitment studies, when *T. chilonis* was recorded attacking *C. pavonana* egg masses, the marginal mortality that it caused ranged from 0.143- 0.474 and in four out of five cases it was the most important egg mortality factor (Table 1). Effective natural enemies of *C. pavonana* are typically lacking in *Brassica* agro-ecosystems (e. g.

Barrion et al., 2003; Morallo-Rejesus et al., 1996; Saucke et al., 2000; Shepard and Schellhorn, 1997) leading to frequent application of insecticides that can disrupt the natural enemies of *P. xylostella* (Furlong et al., 2013). Consequently the discovery of a parasitoid with the potential to suppress *C. pavonana* populations could have significant environmental and economic impacts in Samoa, neighboring Pacific island countries and other regions of the world where *C. pavonana* is an important pest.

Trichogramma chilonis has been recorded from a wide range of Lepidoptera hosts worldwide (Table 2) and in the Asia-Pacific region it has been recorded in China (Liu et al., 2000), Fiji (Hinckley, 1964), Guam (Nafus, 1993), Hawaii (Oatman et al., 1982), Indonesia (Herlinda, 2005), Japan (Ishii, 1941), New Caledonia (Cochereau, 1977), Palau (Doutt, 1955), Papua New Guinea (Saucke et al., 2000), Philippines (Litsinger et al., 2007) and Taiwan (Nagarkatti and Nagaraja, 1971). However, significant taxonomic uncertainty surrounds many species within the genus *Trichogramma* (Noyes et al., 2000) and it is generally accepted that many of the records published before 1970 are unreliable (Polaszek, 2010; Noyes, 2013). Specifically, there has been considerable taxonomic confusion between *T. chilonis*, *T. australicum* and *T. confusum* (Nagarkatti and Nagaraja 1979). Following examination of syntype material of *T. chilonis* Ishii, Nagarkatti and Nagaraja (1979) concluded that the Asian specimens of *T. australicum* that they had described previously (Nagarkatti & Nagaraja 1971) and *T. confusum* Viggiani were younger synonyms of *T. chilonis* Ishii (Ishii 1941). Further, based on examination of the male syntype of *T. australicum* Girault, Pinto et al. (1982) concluded that this species is likely to be endemic to Australia and that published records of *T.*

australicum from outside the continent are probably *T. chilonis.* In many reports "chilonis" has also been incorrectly spelled, compounding the historical taxonomic errors and resulting in further confusion in the literature (Polaszek, 2010). In the present study, specimens were positively identified as *T. chilonis* Ishii based on both morphological characteristics of male genitalia (Nagarkatti and Nagaraja, 1971, 1977, 1979) and ITS2 DNA sequences (Stouthamer et al., 1999). As such we are confident that the parasitoid attacking *C. pavonana* in Samoa conforms to the current conventions that define *T. chilonis*.

Trichogramma chilonis has been recorded in many countries where *C. pavonana* is abundant; e.g. Guam (Nafus and Schreiner, 1986ab), Fiji (Hinckley, 1964), India (Kumar et al., 2009), Indonesia (Herlinda, 2005), Japan (Ishii, 1941; Iga, 1985), Papua New Guinea (Saucke et al., 2000) and Taiwan (Chan and Chou 2000; Nagarkatti and Nagaraja 1971), but it has not yet been recorded causing significant parasitism in the pest anywhere other than Samoa. Indeed in Fiji, extensive sampling of *C. pavonana* eggs has failed to detect any egg parasitoids attacking the pest (Furlong, 2011) and, although *T. chilonis* has been reported attacking *P. xylostella* in Japan (Iga, 1985) and Taiwan (Chan and Chou, 2000; Nagarkatti and Nagaraja, 1971), there are no published records of it attacking *C. pavonana* in these countries. Thus, unraveling the ecological reasons for the success of *T. chilonis* against *C. pavonana* in Samoa will be intriguing and fundamental to any attempts to develop the parasitoid as a biological control agent. Different populations of *T. chilonis* are known to have different efficacies against *P. xylostella* (Tabone et al., 2010) and similar studies to compare the efficacy of the Samoan *T.*

chilonis population with that of other *T. chilonis* populations against *C. pavonana* will be important. Similarly, it will be important to determine the relationship between the Samoan *T. chilonis* population and *P. xylostella*.

The Samoan population of *T. chilonis* appears to have great potential for development as a biological control agent for *C. pavonana*, a pest that has few known natural enemies worldwide. However, considerable further research is required to i) establish the host range of the parasitoid, ii) understand the importance of possible alternative hosts in Samoa (e.g. *Nacoleia octasema* (Meyrick), *Eudocima fullonia (*Clerck), *Hippotion celerio* (L.) and *Agrius convolvuli* (L.), all are common in Samoa (Hopkins, 1928; Kami and Miller, 1998; Sands et al., 1993) and recorded as hosts of *T. chilonis* elsewhere in the Pacific), iii) determine whether thelytokous as well arrhenotokous forms occur within the Samoan population, iv) develop suitable mass production techniques and appropriate release strategies and v) promote the adoption of low insecticide input pest management strategies to encourage *T. chilonis* establishment and maximize its impact on pest populations in commercial crops.

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6. References

Ankersmit, G.W. 1953. DDT-Resistance in *Plutella maculipennis* (Curt.) (Lep.) in Java. Bulletin of Entomological Research 44, 421-425.

Barrion, A.T., Morallo-Rejesus, B., Malabanan-Manipol, J. 2003. New hosts for the parasitoid *Aulacocentrum philippinense* (Ashmead) (Hymenoptera: Braconidae) in Luzon Island, Philippines. The Philippines Entomologist 16, 90-93.

Buchori, D., Meilin, A., Hidayat, P., Sahari, B. 2010. Species distribution of *Trichogramma* and *Trichogrammatoidea* genus (Trichogrammatoidea: Hymenoptera) in Java. J. ISSAAS 16, 83-96.

CABI, 2013. Crop Protection Compendium. Wallingford , UK: CAB International. www.cabi.org/cpc.

Chan M.L., Chou L.Y., 2000. The *Trichogramma* (Hymenoptera: Trichogrammatidae) of Taiwan. Chinese Journal of Entomology 20, 135–151.

Cochereau, P. 1977. Biologie et écologie des populations en Nouvelle-Calédonie d'un papillon piqueur de fruits: *Othries fullonia* Clerck (Lepidoptera, Noctuidae, Catocalinae). Travaux et Documents ORSTOM No. 71, 322 pp.

Doutt, R.L., 1955. Insects of Micronesia. Hymenoptera: Trichogrammatidae and Mymaridae. Insects of Micronesia 19, 1–17.

FAOSTAT. 2013. Production statistics. Rome: FAO. http://faostat.fao.org/site/567/default.aspx#ancor

Furlong, M.J., 2011. Integrated pest management in a sustainable production system for *Brassica* crops in Fiji and Samoa. Annual report PC/2004/063. Australian Centre for International Agricultural Research 33p.

Furlong, M.J., Shi, Z.H., Liu, S.S., Zalucki, M.P., 2004a. Evaluation of the impact of natural enemies on *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) populations on commercial *Brassica* farms. Agricultural and Forest Entomology 6, 311–22.

Furlong MJ, Shi ZH, Liu YQ, Guo SJ, Lu YB, et al. 2004b. Experimental analysis of the influence of pest management practice on the efficacy of an endemic arthropod natural enemy complex of the diamondback moth. Journal of Economic Entomology 97, 1814–1827.

Furlong, M.J., Wright, D. J., Dosdall, L. M. 2013. Diamondback Moth Ecology and Management: Problems, Progress, and Prospects. Annual Review of Entomology 58, 517–541.

Gunn, D., 1925. The larger cabbage moth *Crocidolomia binotalis* Zeller. Journal of Department of Agriculture Union of South Africa 11, 233–239.

Herlinda, S.L. 2005. Fitness variation among *Trichogramma* species/strains on eggs of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Jurnal Perlindungan Tanama 11, 51-59. (Bahasa Indonesia with English title and abstract)

Hinckley, A.D., 1964. Ecological notes on the larvae of some pyralid moths in Fiji. Pacific Science 6, 234–241.

Honda, J. Y. Taylor, L., Rodriguez, J., Yashiro, N. Hirose, Y. 2006. A taxonomic review of the Japanese *Trichogramma* (Hymenoptera: Trichogrammatidae) with descriptions of three new species. Applied Entomology and Zoology 41, 247–267.

Hopkins, G.H.E., 1928. Pests of economic plants in Samoa and other island groups. Bulletin of Entomological Research 18, 23-32.

Iga, M., 1985. The seasonal prevalence of occurrence and the life tables of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). Japanese Journal of Applied Entomology and Zoology 29, 119–125 (in Japanese with English summary).

Ishii, T., 1941. The species of *Trichogramma* in Japan with descriptions of two new species. Kontyû 14, 169–179.

Kami, K.S., Miller, S.E., 1998. Samoan insects and related arthropods: checklist and bibliography. Bishop Museum. Technical Report No. 13, 121 p.

Karungi, J., Lubanga, U.K., Kyamanywa, S., Ekbom, B., 2010. Oviposition preference and offspring performance of *Crocidolomia pavonana* (Lepidoptera: Pyralidae) on different host plants. Journal of Applied Entomology 134, 704–713.

Kumar, G.A., Jalali, S.K., Venkatesan, T., Stouthamer, R., Niranjana, P., Lalitha, Y., 2009. Internal transcribed spacer-2 restriction fragment length polymorphism (ITS-2-RFLP) tool to differentiate some exotic and indigenous trichogrammatid egg parasitoids in India. Biological Control 49, 207–213

Lin, N.Q., 1994. Systematic studies of Chinese Trichogrammatidae. Contributions of the Biological Control Research Institute, Fujian Agricultural University. Special Publication No 4, 362 pp. Chongqing Publishing House, Chongqing, China (Chinese with English summary)

Litsinger, J.A., Dela Cruz, C.G., Canapi, B.L., Barrion, A.T., 2007. Maize planting time and arthropod abundance in southern Mindanao, Philippines. II. Population dynamics of natural enemies. International Journal of Pest Management 53, 161 – 173

Liu, S.S., Wang, X.G., Guo, S.J., He, J.H., Shi, Z.H., 2000. Seasonal abundance of the parasitoid complex associated with the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) in Hangzhou, China. Bulletin of Entomological Research 90, 221–31.

Moore, A., Miller, R.H., 2008. *Daphnis nerii* (Lepidoptera: Sphingidae), a new pest of Oleander on Guam, including notes on plant hosts and egg parasitism. Proceedings of the Hawaiian Entomological Society 40, 67–70.

Morallo-Rejesus, B., Navasero-Ward, R., 2003. Biology of *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) and population interventions against this recurrent pest of crucifers in the Philippines. The Philippines Entomologist 17, 141-168

Morallo-Rejesus, B., Javier, P.A., Barile, V. R., 1996. Exploration and evaluation of the egg parasitoids of *Crocidolomia binotalis* in the Philippines. Proceedings of the AVNET-II final workshop, 1-6 Sept. 1996, Bangkok, Thailand, pp 300-304.

Muniappan R., Marutani M., 1992. Pest management for head cabbage production on Guam. In: Talekar NS (Ed.) Diamondback moth and other crucifer pests: Proceedings of the Second International Workshop, 10–14 December 1990, Tainan, Taiwan. AVRDC Publication No. 92-368. Asian Vegetable Research and Development Center, Taipei, pp. 419–426.

Nafus, D.M., 1993. Movement of introduced biological control agents onto nontarget butterflies, *Hypolimnas* spp. (Lepidoptera: Nymphalidae). Environmental Entomology 22, 265-272.

Nafus, D., Schreiner, I., 1986a. Intercropping maize and sweet-potatoes - effects on parasitization of *Ostrinia furnacalis* eggs by *Trichogramma chilonis*. Agriculture Ecosystems & Environment 15, 189-200.

Nafus, D.M., Schreiner, I.H., 1986b. Parasitoids of the corn borer *Ostrinia furnacalis* (Lep.: Pyralidae) in the Mariana Islands. Entomophaga 31, 219–224.

Nagarkatti, S., Nagaraja, H., 1971. Redescriptions of some known species of *Trichogramma*, showing the importance of the male genitalia as a diagnostic character. Bulletin of Entomological Research 61, 13–31.

Nagarkatti, S., Nagaraja, H., 1977. Biosystematics of *Trichogramma* and *Trichogrammatoidea* species. Annual Review of Entomology 22, 157-76.

Nagarkatti, S., Nagaraja, H., 1979. The status of *Trichogramma chilonis* Ishii (Hym: Trichogrammatidae). Oriental Insects 13, 115-117.

Noyes, J.S. 2013. Universal Chalcidoidea Database. World Wide Web electronic

publication. http://www.nhm.ac.uk/chalcidoids

Noyes, J.S., Pinto, J.D., Stouthamer, R., 2000 *Trichogramma evanescens*: one of the best known species of parasitic Hymenoptera, or is it? Abstracts. Antonie van Leeuwenhoek Symposium. 7th European workshop on insect parasitoids, 1–6 October 2000, Teylers museum, Haarlem, The Netherlands. Wageningen University, The Netherlands.

Oatman, E.R., Pinto, J.D., Platner, G.R. 1982. *Trichogramma* (Hymenoptera: Trichogrammatidae) of Hawaii. Pacific Insects 24, 1–24.

Pinto, J.D., Oatman, E.R., Platner, G.R., 1982. *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae): Redescription and lectotype designation. Pan–Pacific Entomology 58, 48–52.

Polaszek, A. 2010. Species Diversity and Host Associations of *Trichogramma* in Eurasia. In Consoli, F.L., Parra, J.R.P., Zucchi, R.A. (Eds.), Egg Parasitoids in Agroecosystems with Emphasis on *Trichogramma*, Progress in Biological Control 9, 237–266.

Sands, D.P.A., Liebregts, W.J.M.M., Broe, R.J., 1993. Biological control of the fruit piercing moth, *Othreis fullonia* (Clerck) (Lepidoptera: Noctuidae) in the Pacific. Micronesica 4, 25-31.

Sastrosiswojo, S., Setiawati, W., 1992. Biology and control of *Crocidolomia binotalis* in Indonesia. In: Talekar N.S. (ed.) Proceedings of the Second International Workshop on Diamondback Moth and Other Crucifer Pests. Tainan, Taiwan: Asian Vegetable Research and Development Center. pp. 81-87.

Saucke, H., Dori, F., Schmutterer, H., 2000. Biological and integrated control of *Plutella xylostella* (Lep., Yponomeutidae) and *Crocidolomia pavonana* (Lep., Pyralidae) in *Brassica* crops in Papua New Guinea. Biocontrol Science and Technology 10, 595–606.

Shepard, B.M., Schellhorn, N.A., 1997. A *Plutella*/ *Crocidolomia* management program for cabbage in Indonesia. In: Sivapragasam, A., Loke, W.H., Hassan, A.K., Lim, G.S. (Eds.) The Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Third International Workshop, Kuala Lumpur, Malaysia, 29 October - 1 November 1996. Kuala Lumpur, Malaysia: Malaysian Agricultural Research and Development Institute. pp. 262-266.

Singh, O.P., Rawat, R.R., 1980. Natural enemies of cabbage webworm, *Crocidolomia binotalis* Zell., at Jabalpur (Madhya Pradesh). Indian Journal of Entomology 42, 324-325.

Singh, O.P., Rawat, R.R., 1983. Bionomics of the cabbage web-worm, *Crocidolomia binotalis* Zell. on mustard at Jabalpur. Bulletin of Entomology 24, 75-82.

Smee, C., 1942. Report for the Entomologists 1941. Zombo Department of Agriculture, Nyasaland.

Smyth, R.R., Hoffmann, M.P., Shelton, A.M., 2003. Larval performance in relation to labile oviposition preference of *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) among phenological stages of cabbage. Environmental Entomology 32, 765–770.

Srinivasan, K., Veeresh, G. K. 1986. The development and comparison of visual damage thresholds for the chemical control of *Plutella xylostella* and *Crocidolomia binotalis* on cabbage in India. Insect Science and its Application 7, 547-557.

Stouthamer, R., Jianguo Hu, J. G., Van Kan, F.J.P.M., Platner, G.R., Pinto, J.D., 1999. The utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma*. *BioControl* 43, 421–440.

Tabone, E., Bardon, C., Desneux, N., Wajnberg, E., 2010. Parasitism of different *Trichogramma* species and strains on *Plutella xylostella* L. on greenhouse cauliflower. Journal of Pest Science 83, 251–256

Takeuchi, H., Zalucki, M. P., Furlong, M. J. 2009 *Crocidolomia pavonana* larval foraging: behavior and feeding site preferences on cabbage, *Brassica oleracea*. Entomologia Experimentalis et Applicata 133, 154–164.

Wang, D.S., He, Y.R. Guo, X.L., Luo. Y. L. 2012. Acute toxicities and sublethal effects of some conventional insecticides on *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Journal of Economic Entomology 105, 1157-1163.

Waterhouse, D.F., Norris K.R., 1987. Biological control – Pacific prospects. Inkata Press, Melbourne, 454p.

Table 1: Partial life-tables for *C. pavonana* eggs recruited to cabbage crops at Nu'u Crops Research Centre, Samoa (September 2007- December 2009)[a]

Table 2: Known Lepidopteran host range of *Trichogramma chilonis* based on published papers.

Study date [n= plants surveyed]	Stage	Factor	lx [b]	dx	Marginal rate	k-value
September 2007	Egg		3672			
[n=398]		disappearance		432	0.118	0.054
		T. chilonis		1294	0.399	0.221
	Neonate		1946			
August 2008	Egg		529			
[n=198]		disappearance		230	0.435	0.248
		T. chilonis		0	0.000	0.000
	Neonate		299			
November 2008	Egg		1362			
[n=198]		disappearance		72	0.053	0.023
		T. chilonis		450	0.349	0.186
	Neonate		840			
May 2009	Egg		1288			
[n=100]		disappearance		1081	0.839	0.793
		T. chilonis		0	0.00	0.000
	Neonate		207			
November 2009	Egg		641			
[n=100]		disappearance		256	0.400	0.222
		T. chilonis		55	0.143	0.067
	Neonate		329			
Early December 2009	Egg		571			
[n=36]		disappearance		252	0.441	0.253
		T. chilonis		151	0.474	0.279
	Neonate		168			
Mid December 2009	Egg		502			
[n=36]		disappearance		0	0.000	0.000
		T. chilonis		74	0.148	0.070
	Neonate		428			

[a] Marginal rates of egg disappearance and *T. chilonis* parasitism were calculated using the methods described by Furlong et al. (2004b).

[b] It was not possible to accurately count the number of eggs in an egg mass in the field. When surviving egg masses were taken to the laboratory, the number of eggs in each was determined under a microscope, mean numbers of eggs in field-collected egg masses were as follows: September 2007= 21.3 (±1.9); August 2008= 23.0 (±2.1); November 2008= 23.9 (±2.3); May 2009= 23.0 (±2.9); November 2009= 18.3 (±3.1); Early December 2009= 16.8 (±3.2); Mid December 2009= 18.6 (±2.0). These means were used to estimate egg numbers recruited to the field based on the number of egg masses recruited during each defined time period.

Family	Total records (% of total)	Species[a]
Arctiidae	3 (2)	*Amsacta moorei*[1], *Spilarctia obliqua*[1], *Spilosoma obliqua*[1]
Blastobasidae	1 (<1)	*Pseudohypatopa pulverea*[1]
Cossidae	1 (<1)	*Phragmataecia gummata*[1]
Crambidae	32 (22)	*Chilo auricilius*[1], *Chilo indicus*[1], *Chilo infuscatellus*[2], *Chilo partellus*[2], *Chilo sacchariphagus*[2], *Chilo simplex*[3], *Chilo suppressalis*[2], *Chilo venosatus*[2], *Cnaphalocrocis medinalis*[2], *Crocidolomia pavonana*[4], *Deanolis sublimbalis*[1], *Diaphania indica*[1], *Diatraea saccharalis*[1], *Diatraea* sp.[3], *Emmalocera depressella*[2], *Eoreuma loftini*[1], *Hymenia recurvalis*[2], *Leucinodes orbonalis*[5], *Marasmia patnalis*[1], *Marasmia exigua*[5], *Nacoleia octasema*[6], *Omiodes indicata*[1], *Ostrinia furnacalis*[7], *Ostrinia nubilale*[1], *Parapoynx stagnalis*[1], *Psara sp.* [1], *Scirpophaga incertulas*[1], *Scirpophaga intacta*[1], *Scirpophaga nivella*[1], *Scirpophaga excerptalis*[1], *Scirpophaga* sp.[2] *Tryporyza incertulas*[2]
Danaidae	1 (<1)	*Anosia chrysippus*[1]
Eupterotidae	1 (<1)	*Apha aequalis*[1]
Gelechiidae	3 (2)	*Pectinophora gossypiella*[1], *Phthorimaea operculella*[1], *Sitotroga cerealella*[1], *Boarmia variegata*[1]
Gracillariidae	1 (<1)	*Caloptilia* sp.[2]
Hesperiidae	3 (2)	*Parnara guttata*[1], *Pelopidas mathias*[2], *Udaspes folus*[8]
Hyblaeidae	1 (<1)	*Hyblaea puera*[1]
Lasiocampidae	2 (1)	*Dendrolimus punctatus*[1], *Gastropacha* sp.[1]
Lycaenidae	6 (4)	*Deudorix epijarbas*[1], *Jamides bochus formosanus*[8], *Lampides boeticus*[8], *Virachola isocrates*[1], *Virachola livia*[1], *Zizeeria maha argia*[5]
Lymantriidae	3 (2)	*Euproctis similis*[5], *Ivela auripes*[1], *Orgyia postica*[8]

Noctuidae	32 (22)	*Achaea janata*[2,10], *Anomis flava*[1], *Arcte coerulea*[2], *Asota ficus*[5], *Autographa nigrisigna*[5], *Busseola fusca*[1], *Chrysodeixis chalcites*[1], *Earias insulana*[1], *Earias sp.*[1], *Earias vittella*[1], *Eublemma amabilis*[1], *Eudocima fullonia*[9], *Helicoverpa armigera*[2], *Helicoverpa assulta*[2,5], *Helicoverpa zea*[10], *Heliothis sp.*[1], *Heliothis virescens*[1], *Mammestra brassicae*[5], *Naranga aenescens*[1], *Othreis sp.*[1], *Plusia nigrisigna*[1] *Plusia orichalcea*[1] *Rivula atimeta*[1] *Sesamia calamistis*[2] *Sesamia inferens*[2] *Spodoptera exigua*[1] *Spodoptera litura*[2, 5] *Spodoptera mauritia*[10] *Spodoptera sp.*[1] *Tiracola plagiata*[2] *Tiracola plagiata*[1] *Trichoplusia ni*[2, 8]
Notodontidae	1 (<1)	*Clostera cupreata*[1]
Nymphalidae	6 (3)	*Acraea issoria*[8], *Agraulis vanillae*[10], *Danaus chrysippus chrysippus*[5, 8], *Danaus plexippus*[10], *Hipolimnas anomala*[1], *Hipolimnas bolina*[1], *Hypolimnas anomala*[7], *Hypolimnas bolina*[7], *Melanitis leda*[1]
Oecophoridae	1 (<1)	*Opisina arenosella*[1]
Papilionidae	5 (4)	*Papilio demoleus libanius*[8], *Papilio machaon hippocrates*[5], *Papilio polytes*[1], *Papilio protenor demetrius*[5], *Papilio xuthus*[5, 10]
Pieridae	4 (3)	*Catopsilia pyranthe*[1], *Eurema sp.*[1], *Pieris brassicae*[1], *Pieris rapae*[1]
Plutellidae	1 (<1)	*Plutella xylostella*[5,8]
Pyralidae	4 (3)	*Corcyra cephalonica*[1], *Corcyra sp.*[1], *Ephestia kuehniella*[1], *Raphimetopus ablutellus*[1]
Saturniidae	6 (4)	*Antheraea pernyi*[1], *Antheraea yamamai*[1], *Dictyoploca japonica*[1], *Philosamia cynthia*[1], *Philosamia ricini*[1], *Samia cynthia*[1], *Acherontia styx*[1]
Sphingidae	9 (6)	*Agrius cingulata*[10], *Agrius convolvuli*[2,5,11], *Cephanodes hylas*[5], *Clanis bilineata*[1], *Daphnis nerii*[10,12], *Hippotion celerio*[13] *Macroglossum pyrrhostictum*[8,10] *Theretra silhetensis*[5]
Tortricidae	13 (9)	*Adoxophyes orana*[1], *Argyroploce schistaceana*[1], *Bactra* sp.[2], *Bactra truculenta*[1], *Cophoprora sp.*[1], *Cydia pomonella*[1], *Eucosma schistaceana*[1], *Homona coffearia*[2], *Laspeyresia koenigana*[1], *Leguminivora glycinivorella*[5], *Pandemis heparana*[1], *Peronea crocepepla*[1], *Tetramoera schistaceana*[2]

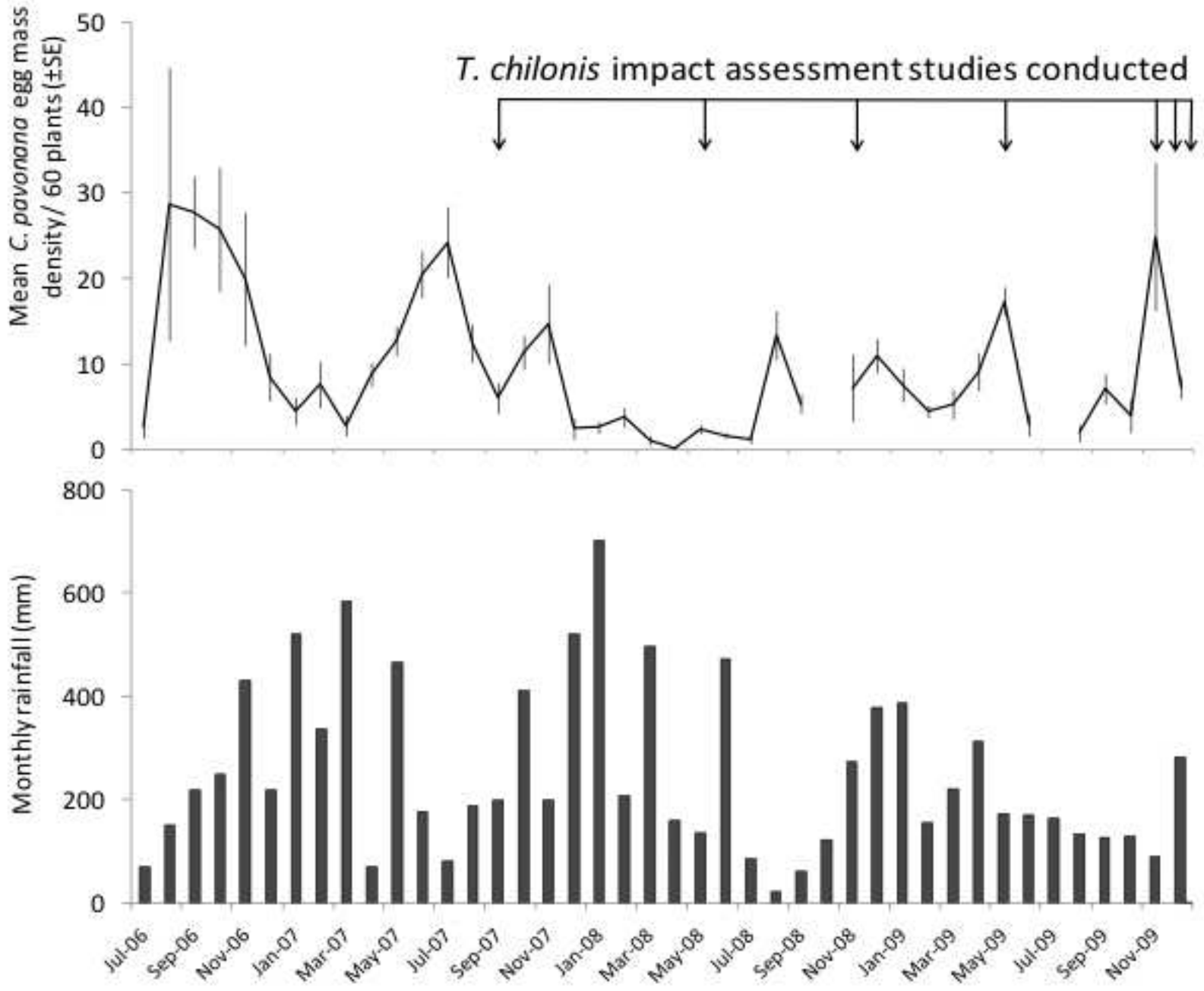
[a] Numbers refer to reference source: [1]Polaszeck (2010), [2]Nagarkatti and Nagaraja (1971), [3]Ishii (1941), [4]Saucke et al. (2000), [5]Honda (2006), [6]Hinckley (1964), [7]Nafus (1993), [8]Chan and Chou (2000), [9]Cochereau (19770, [10] Oatman et al (1992), [11]Nafus and Schreiner (1986), [12]Moore and Miller (2008), [13]Doutt (1955).

Figures

Figure 1: Seasonal abundance of *Crocidolomia pavonana* egg masses in sequentially planted cabbage crops at Nu'u Crops Research Centre, July 2006- December 2009.

Figure 2: Locations of *Brassica* farms on Upolu, Samoa that were surveyed for *C. pavonana* egg masses and *T. chilonis* June 2011- November 2013.

Figure 3: Male genitalia of *Trichogramma chilonis* Ishii (x1000 magnification) collected from *C. pavonana* in Samoa in 2007. Dorsal expansion of gonobase (DEG) is triangular, highly sclerotized and there are prominent lateral lobes (LL) and a round posterior extremity extending to half the length of D (=length between the base of the median ventral projection (MVP) and the apex of gonoforceps (GF)). MVP is broad and triangular and extends approximately one-third the length of D. Chelate structures (CS), extend to approximately half of D.



Leauvaa (elevation:22m)

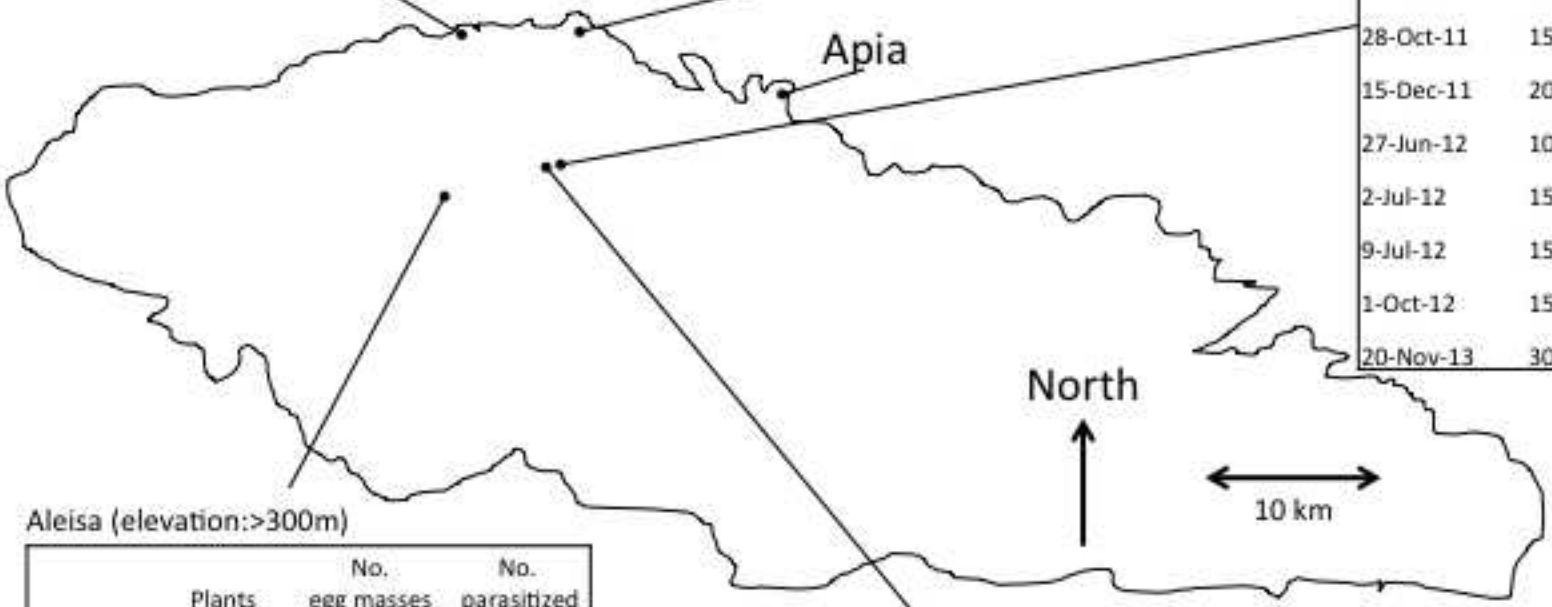
Date	Plants sampled	No. egg masses (per plant)	No. parasitized (%)
14-Jun-12	100	19 (0.19)	1 (5)
15-Jun-12	150	19 (0.13)	0 (0)
16-Aug-13	300	48 (0.16)	9 (19)

Faleula (elevation:15m)

Date	Plants sampled	No. egg masses (per plant)	No. parasitized (%)
15-Jan-12	150	30 (0.20)	0 (0)
16-Aug-13	200	58 (0.29)	17 (29)
8-Oct-13	300	64 (0.21)	6 (9)

Atele and Chinese Farms (elevation:100m)

Date	Plants sampled	No. egg masses (per plant)	No. parasitized (%)
2-Aug-11	300	150 (0.50)	23 (15)
25-Sep-11	200	60 (0.30)	29 (48)
27-Oct-11	150	35 (0.23)	0 (0)
27-Oct-11	200	35 (0.18)	15 (43)
28-Oct-11	150	25 (0.17)	0 (0)
15-Dec-11	200	61 (0.31)	11 (18)
27-Jun-12	100	19 (0.19)	0 (0)
2-Jul-12	150	31 (0.21)	2 (7)
9-Jul-12	150	30 (0.20)	0 (0)
1-Oct-12	150	30 (0.20)	0 (0)
20-Nov-13	300	62 (0.21)	10 (16)

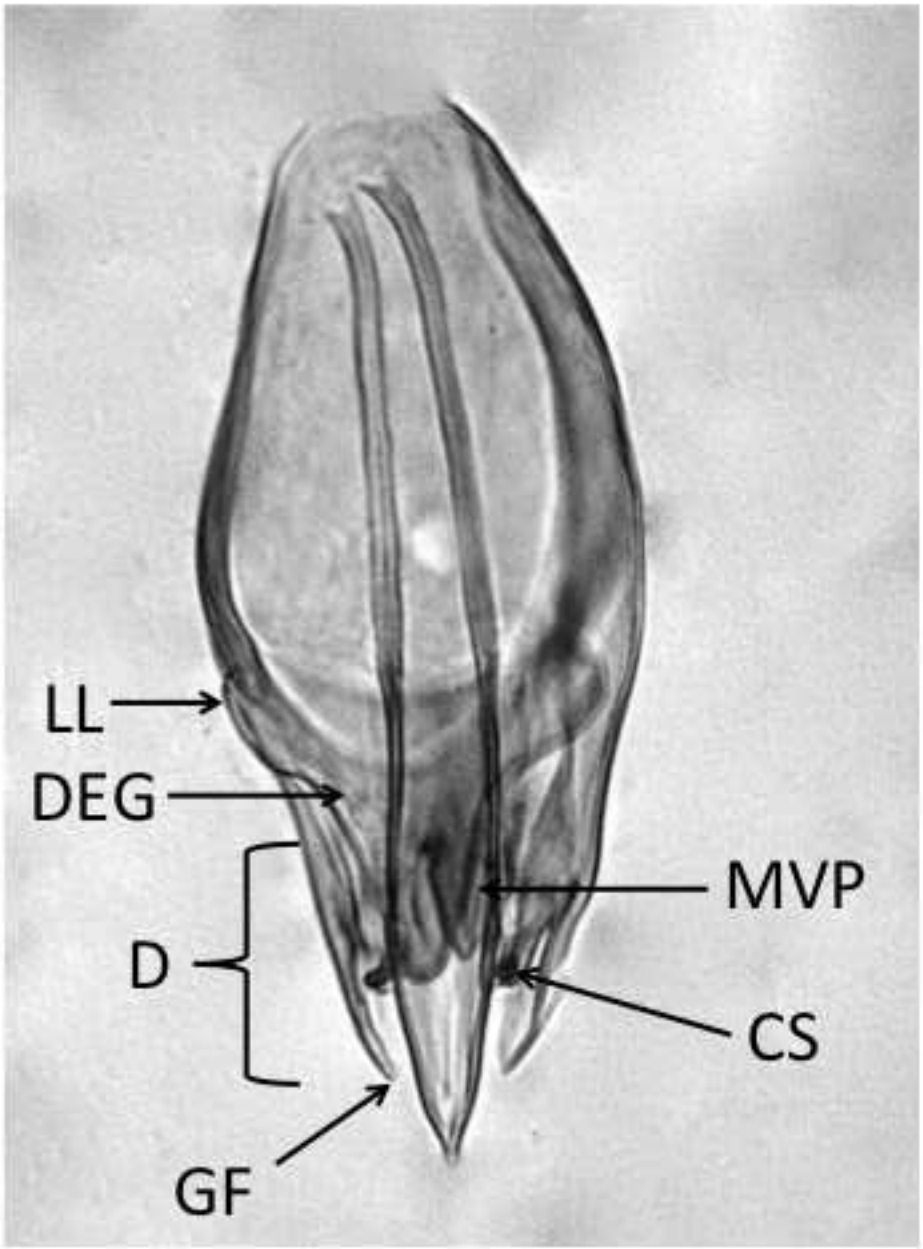


Aleisa (elevation:>300m)

Date	Plants sampled	No. egg masses (per plant)	No. parasitized (%)
26-Jul-11	200	120 (0.60)	22 (18)
5-Jan-12	150	20 (0.13)	3 (15)
10-Apr-12	150	70 (0.47)	10 (14)
5-Jun-12	200	31 (0.16)	0 (0)
3-Apr-13	200	20 (0.10)	0 (0)
29-May-13	200	20 (0.10)	0 (0)
18-Jun-12	100	10 (0.10)	1 (10)

Nu'u Crop Center (elevation:75m)

Date	Plants sampled	No. egg masses (per plant)	No. parasitized (%)
18-Jun-11	200	89 (0.45)	77 (87)
17-Aug-12	150	60 (0.40)	17 (28)
2-Sep-12	150	30 (0.20)	0 (0)
19-Sep-12	200	50 (0.25)	2 (4)



- We report *Trichogramma chilonis* Ishii parasitizing *Crocidolomia pavonana* F. in Samoa
- This is the first record of *T. chilonis* in Samoa
- It is widely established and can significantly suppress *C. pavonana* populations
- The parasitoid has significant potential for biological control of *C. pavonana* elsewhere